Effects of Replacing Fish Meal with Fermented Soybean Meal on Growth Performance, Body Composition, Serum Biochemical Indices, and Liver Histomorphology in Juvenile Large Yellow Croaker (Larimichthys crocea) Postprint

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Abstract

This experiment aimed to investigate the effects of replacing fish meal with fermented soybean meal on the growth performance, body composition, serum biochemical indices, and liver histomorphology of juvenile large yellow croaker (Larimichthys crocea), and to explore the appropriate proportion of fish meal replacement by fermented soybean meal in the diet of juvenile large yellow croaker. Using fish meal and wheat gluten meal as the main protein sources, and fish oil, soybean oil, and soybean lecithin as the main lipid sources, a basal diet containing 40% fish meal was formulated. Fermented soybean meal was used to replace 0 (R0 group, as control), 15% (R15 group), 30% (R30 group), 45% (R45 group), 60% (R60 group), and 75% (R75 group) of the fish meal in the basal diet, and appropriate amounts of crystalline amino acids (lysine and methionine) were added to all diets except the control group diet to formulate six isonitrogenous (crude protein level of 45%) and isolipidic (crude lipid level of 10%) experimental diets. The feeding trial was conducted in seawater cages (1.5 m$\times$1.5$m\times$2.0m), $withthreecagesforeachexperimentaldiet, andeachcagewasstockedwith60juvenilelargeyello$ g. The feeding trial lasted for 56 days. The results showed that there were no significant differences in the survival rate of juvenile large yellow croaker among all groups (P>0.05), but there was a decreasing trend with the increase of fish meal replacement ratio by fermented soybean meal. Compared with the R0 group, the specific growth rate (SGR), weight gain rate (WGR), and feed conversion ratio (FCR) of the R15, R30, and R45 groups showed no significant differences (P>0.05). With further increase in fish meal replacement ratio (R60 and R75 groups), SGR and WGR decreased significantly (P<0.05), while FCR increased significantly (P<0.05). There were no significant differences

in whole-body crude protein, crude lipid, and moisture content among all groups (P>0.05). With the increase of fish meal replacement ratio by fermented soybean meal, whole-body crude ash content showed an increasing trend. There were no significant differences in serum biochemical indices among all groups (P>0.05), but with the increase of fish meal replacement ratio by fermented soybean meal, serum total cholesterol content showed a decreasing trend, while alanine aminotransferase activity showed an increasing trend. Liver histological observation revealed that fish meal replacement ratio by fermented soybean meal exceeding 30% caused damage to hepatocytes, and the higher the replacement ratio, the more severe the damage. Based on comprehensive evaluation of all measured indices, this study suggests that replacing 30% of fish meal in the diet (containing 40% fish meal) with fermented soybean meal is appropriate, and excessive replacement ratio would cause liver tissue lesions in juvenile large yellow croaker, leading to decreased growth rate and survival rate.

Full Text

Effects of Replacement of Fish Meal by Fermented Soybean Meal on Growth Performance, Body Composition, Serum Biochemical Indices and Liver Tissue Morphology of Juvenile Large Yellow Croaker (Larimichthys crocea)

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Abstract: This experiment investigated the effects of replacing fish meal with fermented soybean meal (FSM) on growth performance, body composition, serum biochemical indices, and liver tissue morphology in juvenile large yellow croaker (*Larimichthys crocea*), aiming to determine the optimal replacement proportion. A basal diet containing 40% fish meal was formulated using fish meal and wheat gluten meal as primary protein sources, and fish oil, soybean oil, and soybean lecithin as primary lipid sources. Six isonitrogenous (45% crude protein) and isolipidic (10% crude lipid) experimental diets were prepared by replacing 0% (R0, control), 15% (R15), 30% (R30), 45% (R45), 60% (R60), and 75% (R75) of fish meal with FSM. Crystalline amino acids (lysine and methionine) were supplemented in all diets except the control to eliminate limiting amino acid effects. The feeding trial was conducted in seawater cages (1.5 m × 1.5 m × 2.0 m) with three replicate cages per diet, each stocked with 60 juvenile fish (initial weight 10.49 ± 0.03 g) for 56 days. Results showed no significant differences in survival rate among groups (P > 0.05), though a declining trend was observed with increasing FSM substitution. Specific growth rate (SGR),

weight gain rate (WGR), and feed conversion ratio (FCR) in R15, R30, and R45 groups did not differ significantly from R0 ($P > 0.05$). However, higher replacement levels (R60 and R75) significantly decreased SGR and WGR ($P < 0.05$) and increased FCR ($P < 0.05$). Whole-body crude protein, crude lipid, and moisture contents showed no significant differences among groups ($P > 0.05$), while ash content exhibited an upward trend with increasing FSM substitution. Serum biochemical indices did not differ significantly among groups ($P > 0.05$), though total cholesterol tended to decrease and alanine aminotransferase activity tended to increase with higher replacement levels. Histological observation revealed that FSM replacement exceeding 30% caused liver cell damage, with severity increasing at higher substitution rates. Based on comprehensive evaluation, replacing 30% of fish meal in diets containing 40% fish meal with FSM is appropriate for juvenile large yellow croaker; higher replacement levels cause liver pathological changes and reduce growth and survival.

Keywords: juvenile large yellow croaker; fermented soybean meal; growth performance; body composition; serum biochemical indices; liver tissue morphology

Introduction

Fish meal constitutes a primary component of aquafeeds, typically accounting for over half of feed costs. With rapid development of aquaculture and increasing demand for fish meal, coupled with declining marine fishery resources, fish meal prices continue to rise. To alleviate supply-demand contradictions, reduce feed costs, and improve economic benefits, extensive research on fish meal replacement has been conducted worldwide. These studies demonstrate that using alternative inexpensive protein sources to replace fish meal in feeds is feasible. Soybean meal is the most widely used plant protein source in aquafeeds, but its application is limited by various anti-nutritional factors that hinder fish digestion and absorption, restricting its replacement level for fish meal. Microbial fermentation of soybean meal can effectively decompose and destroy these anti-nutritional factors while improving nutritional value. Studies on grouper (*Epinephelus coioides*), Pacific white shrimp (*Penaeus vannamei*), gibel carp (*Carassius auratus gibelio*), and rainbow trout (*Oncorhynchus mykiss*) have confirmed that fermented soybean meal outperforms conventional soybean meal in aquafeed applications.

Large yellow croaker (*Larimichthys crocea*), belonging to Osteichthyes, Perciformes, Sciaenidae, and genus *Larimichthys*, is one of the traditional "Four Major Seafoods" and a major mariculture economic fish in China. Following successful artificial breeding, large yellow croaker has become a primary marine aquaculture species in China. To address high fish meal prices and supply shortages, domestic scholars have conducted fish meal replacement studies in formulated feeds, though no reports on fermented soybean meal replacement have been published. Therefore, this experiment investigated the effects of replac-

ing different proportions of fish meal with fermented soybean meal on growth performance, body composition, serum biochemical indices, and liver tissue morphology in juvenile large yellow croaker to determine optimal replacement levels, enrich nutritional databases for large yellow croaker feeds, and provide theoretical support for developing complete formulated feeds.

1.1 Experimental Design and Diets

A basal diet containing 40% fish meal was formulated using fish meal (Peruvian) and wheat gluten meal as primary protein sources, and fish oil, soybean oil, and soybean lecithin as primary lipid sources. Six isonitrogenous (45% crude protein) and isolipidic (10% crude lipid) experimental diets were prepared by replacing 0% (R0, control), 15% (R15), 30% (R30), 45% (R45), 60% (R60), and 75% (R75) of fish meal with fermented soybean meal (FSM, purchased from Ningbo Tech-Bank Co., Ltd.). Crystalline amino acids (lysine and methionine) were supplemented in all diets except the control to eliminate limiting amino acid effects. The essential amino acid composition of fish meal and FSM is shown in Table 1 , diet formulation and nutrient levels in Table 2 , and dietary amino acid composition in Table 3 . All feed ingredients were ground to pass through an 80-mesh sieve, mixed using the progressive expansion method, and blended with water in a mixer until fully moistened. Pellets were produced using a twin-screw extruder (F-26 , South China University of Technology) and processed into two pellet sizes (2 mm and 4 mm). Pellets were cooked in a 90°C oven for 30 minutes, air-dried, sealed in plastic bags, and stored at -20°C.

1.2 Experimental Fish and Rearing Management

The feeding trial was conducted at Xihu Port, Xiangshan County, Zhejiang Province. Prior to the experiment, juvenile large yellow croaker were acclimated in 3 m × 6 m × 3 m seawater cages for two weeks and fed commercial feed to adapt to formulated diets. After acclimation, healthy fish of uniform size (average weight 10.49 ± 0.03 g) were randomly distributed into five groups with three replicate cages each (1.5 m × 1.5 m × 2.0 m), with 60 fish per cage. Fish were hand-fed to satiation twice daily (05:00 and 17:00) for 56 days. During the trial, water temperature ranged from 25.5 to 29.5°C, salinity from 27‰ to 30‰, and dissolved oxygen remained above 7 mg/L.

1.3 Sample Collection and Analysis

1.3.1 Sample Collection After the 56-day feeding trial, fish were fasted for 24 hours and anesthetized with eugenol (1:1,000) before counting and weighing. Five fish were randomly collected from each cage and blood was drawn from the caudal vein using sterile 2 mL syringes, placed in conventional collection tubes, and allowed to clot at 4°C for 24 hours. Serum was separated by centrifugation (3,000 r/min, 4°C) for 10 minutes and stored at -20°C for biochemical analysis. Three additional fish per cage were sampled for measurement of body length and weight, followed by weighing of viscera and liver to calculate hepatosomatic

index (HSI), viscerosomatic index (VSI), and condition factor (CF). Two fish per cage were dissected to obtain liver samples, which were cleaned of fat and connective tissue, fixed in Bouin' s solution for 24 hours, washed with 70% ethanol, and stored in 70% ethanol for histological examination. Five more fish per cage were stored at -20°C for proximate composition analysis.

1.3.2 Proximate Composition Analysis Proximate composition of feed ingredients, experimental diets, and fish body was determined according to AOAC (1993) methods. Moisture content was measured by drying to constant weight in a 105°C oven. Crude protein content was determined by the semi-micro Kjeldahl method (total nitrogen × 6.25). Crude lipid content was measured by Soxhlet extraction. Ash content was determined by incineration in a muffle furnace at 550°C for 6 hours.

1.3.3 Serum Biochemical Indices Serum biochemical indices were measured using commercial assay kits (Nanjing Jiancheng Bioengineering Institute) following the manufacturer' s instructions.

1.3.4 Liver Histological Observation Liver samples preserved in 70% ethanol were dehydrated through an ethanol gradient, embedded in paraffin, sectioned using a microtome (Leica RM2135), and stained with hematoxylin-eosin (HE) method. Tissue sections were observed under a microscope and photographed.

1.4 Calculation Formulas

Specific growth rate (SGR, %/d) = 100 × (ln Wt - ln Wo) / t
Survival rate (SR, %) = 100 × Nt / No
Weight gain rate (WGR, %) = 100 × (Wt - Wo) / Wo
Feed conversion ratio (FCR) = (T - S) / (Wt - Wo)
Hepatosomatic index (HSI, %) = 100 × Wh / Wt
Viscerosomatic index (VSI, %) = 100 × Wv / Wt
Condition factor (CF, %) = 100 × Wt / L^3

Where: Wo = initial body weight; Wt = final body weight; No = initial number of fish per cage; Nt = final number of fish per cage; T = total feed amount; S = uneaten feed amount; t = experimental duration (56 d); Wh = liver weight; Wv = viscera weight; L = body length.

1.5 Statistical Analysis

Data were analyzed by one-way ANOVA using SPSS 17.0 software. When significant differences were detected, Tukey's multiple comparison test was performed with significance level set at $P < 0.05$. Results are expressed as means ± standard deviation.

Results

2.1 Effects of FSM Replacement on Growth Performance

As shown in Table 4 , no significant differences in survival rate were observed among groups ($P > 0.05$), though a declining trend occurred with increasing FSM substitution. Final body weight, SGR, and WGR in R0 group were significantly higher than those in R60 and R75 groups ($P < 0.05$). The FCR was lowest in R0 group and significantly lower than in R60 and R75 groups ($P < 0.05$). No significant differences in HSI, VSI, or CF were detected among groups ($P > 0.05$) (Table 5).

2.2 Effects of FSM Replacement on Body Composition

Table 6 shows that no significant differences in whole-body crude protein, crude lipid, or moisture contents were found among groups ($P > 0.05$). However, whole-body ash content in R60 and R75 groups was significantly higher than in other groups ($P < 0.05$), with no significant differences among the remaining groups ($P > 0.05$).

2.3 Effects of FSM Replacement on Serum Biochemical Indices

As shown in Table 7 , serum total protein (TP), albumin (ALB), and globulin (GLOB) contents in R0 group did not differ significantly from other groups ($P > 0.05$), though R30 group showed the highest values, significantly higher than R75 group ($P < 0.05$). No significant differences in serum total cholesterol (TCHO), triglycerides (TG), or glucose (GLU) were observed among groups ($P > 0.05$), though TCHO content decreased to varying degrees with FSM substitution. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities showed no significant differences among groups ($P > 0.05$), though ALT activity increased with higher replacement levels.

2.4 Effects of FSM Replacement on Liver Tissue Morphology

Liver histology (Figure 1 [Figure 1: see original paper]) revealed normal hepatocytes in R0, R15, and R30 groups. However, as FSM replacement increased, hepatocyte vacuolization intensified, intracellular fat accumulation worsened (pushing nuclei toward cell membranes), and hepatocyte nuclei gradually dissolved or disappeared with increased cellular disintegration. In R75 group, hepatocyte outlines became indistinct with extensive nuclear loss.

Discussion

3.1 Effects on Growth Performance

Compared with conventional soybean meal, fermented soybean meal exhibits increased crude protein content and improved protein quality, optimizing its nutritional value. Previous studies have indicated that FSM can increase the

proportion of fish meal replacement in feeds. In this study, FSM replacement up to 45% did not affect growth performance, likely because fermentation reduced anti-nutritional factors and increased small peptide content, improving FSM digestibility and thus allowing higher replacement levels. Supplementing diets with crystalline amino acids (lysine and methionine) to eliminate limiting amino acid effects may also contribute to this result. For example, with crystalline amino acid supplementation, FSM can replace up to 40% of fish meal in black sea bream (*Acanthopagrus schlegeli*) diets without affecting growth, and can completely replace fish meal in rainbow trout and *Macrobrachium nipponense* feeds without negative effects. Other studies have reported that phytase or probiotic supplementation can also increase plant protein replacement levels. However, some research shows that growth rate declines with increasing plant protein substitution, which aligns with our results: WGR and SGR decreased with higher FSM replacement, with R60 and R75 groups significantly lower than the control. This may be attributed to reduced feed palatability and feed intake at high FSM inclusion levels. Additionally, FSM replacement had no significant effect on HSI, VSI, or CF, similar to findings in turbot (*Scophthalmus maximus*) fed soy protein concentrate.

3.2 Effects on Body Composition

Studies have reported that FSM replacement does not affect whole-body crude protein content, consistent with our observations. Some research suggests that increasing plant protein levels may reduce body crude lipid content, as observed in turbot and giant grouper (*Epinephelus lanceolatus*), possibly due to intestinal lesions caused by soybean non-starch polysaccharides reducing fat absorption. However, other studies found no significant effects on crude lipid content, consistent with our results. Conversely, some reports indicate that increasing *Chlorella* replacement elevated muscle crude lipid content in gibel carp, while muscle crude protein increased at low substitution levels but decreased at high levels, suggesting different protein sources and lipid metabolism pathways may affect body composition differently. We also observed an upward trend in whole-body ash content with increasing FSM replacement, possibly due to increased calcium or phosphorus deposition, though the mechanism requires further investigation.

3.3 Effects on Serum Biochemical Indices

Serum TP synthesized in the liver can indicate liver damage, as structural changes affect TP content. In this study, FSM replacement did not significantly affect serum TP, ALB, or GLOB levels. Some studies suggest plant protein substitution may affect energy metabolism, decreasing serum TCHO and TG with increasing replacement, consistent with our TCHO results, possibly due to soybean flavonoids in FSM. Normally, ALT resides in hepatocytes while AST is primarily in hepatic mitochondria, with low serum activities that increase only when membrane permeability increases or cells necrose. Although no signifi-

cant differences in AST or ALT were observed, the increasing ALT trend with higher FSM replacement suggests liver tissue damage, indicating excessive FSM substitution may cause hepatic injury.

3.4 Effects on Liver Tissue Morphology

Studies have shown that dietary plant protein reduces liver physiological function; during liver disease, lipoprotein synthesis decreases and hepatic fat cannot be transported efficiently, leading to fat accumulation. Elevated dietary lipid levels also cause hepatic fat deposition, potentially inducing fatty liver. Research on Japanese seabass (*Lateolabrax japonicus*) found severe hepatocyte damage and obvious fatty degeneration at high replacement levels (60% and 80%). Our histological observations showed normal liver tissue in R0, R15, and R30 groups, but increasing FSM replacement intensified hepatocyte vacuolization, nuclear dissolution, and liver pathology, consistent with findings in gibel carp. Therefore, appropriate plant protein inclusion levels should be carefully considered in aquaculture practice.

In conclusion, replacing 30% of fish meal with fermented soybean meal in diets containing 40% fish meal is suitable for juvenile large yellow croaker, while excessive replacement causes liver pathological changes and reduces growth and survival rates.

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Note: Figure translations are in progress. See original paper for figures.